

## EXTRACTIVE METHOD DEVELOPMENT AND VALIDATION OF TULOButEROL IN API AND ITS PHARMACEUTICAL DOSAGE FORMS BY SPECTROPHOTOMETRY

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### Abstract

A simple, accurate, sensitive and reproducible visible spectrophotometric method has been developed for the determination of Tulobuterol (TLB) in bulk and also in its pharmaceutical dosage formulations. The proposed method was based on complexation of the drug with Bromo Cresol Green (BCG) extracted with chloroform showing absorbance maxima at 624 nm respectively. Beer's law is obeyed over a concentration range of 0.1-0.8 µg/mL. The respective linear regression equation being  $Y (0.0309) = 1.065x + 0.0396$  for TLB. Results of analysis for the method established, was validated statistically and also by recovery studies. The color was stable for about 1½ hour. The apparent molar absorptivity and Sandell's Sensitivity values are  $0.43 \times 10^4 \text{ L}^{\text{mol}^{-1}} \text{cm}^{-1}$  and  $0.7674 \mu\text{gcm}^{-2}$  respectively. The assay and recovery studies were found to be 101.43% and coefficient correlation (r) was found to be 0.994. The different experimental parameters effecting the development and stability were studied carefully and optimized. No interference was observed in the presence of common pharmaceutical excipients. The validity of the methods was tested by analyzing the drug in its pharmaceutical preparations. Good recoveries were also obtained. The developed method employed was successful for the determination of TLB in various pharmaceutical preparations.

**Keywords:** TLB, BCG, Visible Spectrophotometric Method, Molar Absorptivity & Sandell's Sensitivity

### 1. Introduction

Tulobuterol (TLB) (Empirical Formula:  $\text{C}_{12}\text{H}_{16}\text{ClNO}$ , Mol.Weight: 227.730 g/mol) chemically is, (RS)-2-(tert-butylamino)-1-(2-chlorophenyl) Ethanol.

(Figure: 1). Chronic Obstructive Pulmonary Disease (COPD) is an inflammatory lung disease that occurs as a result of inhalation of harmful particles, such as those in cigarette smoke. There is some concern that the number of COPD patients will increase with the aging of the population. The global initiative for Chronic Obstructive Lung Disease (COLD) recommends the use of long-acting bronchodilators<sup>1-5</sup>, such as anti cholinergics,  $\beta_2$  adrenergic receptor agonists and methyl xanthenes for the management of stable COPD patients. TLB is a direct-acting sympathomimetic with selective action on  $\beta_2$ -receptors. Thus, TLB is a selective  $\beta_2$  adrenergic agonist with minimal nonselective inhibitory effect<sup>6-9</sup> on airway and vascular smooth muscle. It also facilitates adrenergic neurotransmission, which may help to explain its bronchodilator effect in the intact organism. TLB does not activate  $\beta_1$  adrenoceptors and has no direct positive chronotropic effect<sup>10-13</sup>. As highlighted earlier, the use of the above drug has become, very wide spread. The survey of literature showed a very few chromatographic methods and biological analytical methods<sup>14-18</sup> irrespective of any single spectrophotometric method for the analysis of selected drug at the time of commencement of these investigations. So in order to bridge this gap the authors are fascinated in choosing this drug. A detailed account of all analytical methods existing for the drug is made to avoid duplication of the method

developed. The authors has made some humble attempts, hoping to fulfill and bridge this gap, in succeeding the developed extractive analytical method by using spectrophotometry, verifying the efficacy and safety of TLB. The results of this labor of love are set forth in this article.

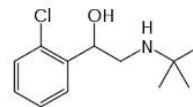


Figure 1: Tulobuterol

### 2. Experimental:

#### 2.1 Materials & methods

**2.1.1 Instrument:** Shimadzu double beam Ultra Violet –Visible Spectrophotometer UV-1800 with 1 cm matched quartz cells were used for all spectral measurements.

**2.1.2 Chemicals & Reagents:** All the chemicals used were of analytical & extra pure reagent grade, procured from SD Fine Chemicals (SDFC), Mumbai, India. All the solutions were freshly prepared.

1. Acid Phthalate Buffer pH 2.4
2. Bromo Cresol Green-BCG (0.1%)
3. Chloroform AR grade
4. Distilled Water
5. Hydrochloric Acid
6. Methanol AR grade
7. Potassium Hydrogen Phthalate EP

#### 2.2 Preparative Analytical Methodology

**2.2.1 Preparation of Phthalate buffer pH 2.4:** Add 250 ml of 0.2 M potassium Hydrogen Phthalate to 10

ml of 0.2 M HCL make up the final volume with water to produce 1000 ml.

**2.2.2 Preparation of 0.1% Bromo Cresol Green (BCG) dye solution:** Weigh 0.1 gm of dye sample and dissolve in small quantity of distilled water and make up to 100 ml and filter it using filter paper if necessary.

**2.2.3 Procedure**

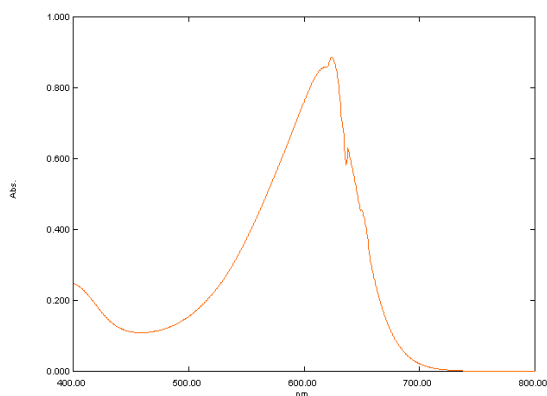
**2.2.3.1 Preparation of standard stock and working sample solution:**

Weigh 0.5 gm of bulk drug (TLB) and dissolve in 50 ml of methanol, shake well till it dissolves and make up to 100 ml with the same. From the above stock solution, working sample solution was prepared from 0.1-0.8 ml (1-8/ml) respectively.

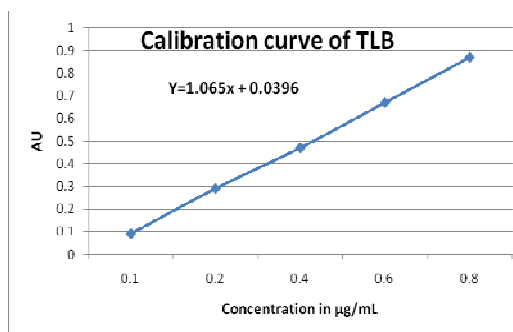
**2.2.3.2 Assay**

Aliquots of standard drug solution of TLB containing 0.1-0.8 ml (1-8 µg/mL) were taken and transferred into series of separating funnels. To each separating funnel, 2 ml of BCG, 2 ml of Phthalate buffer pH 2.4 and 5 ml of chloroform were added. The contents are shaken thoroughly for 5 min and allowed to stand for 15 minutes, so as to separate the aqueous and chloroform layer for the formation of colored ion association complex. The absorbance of the yellow colored chromogen was measured at 624 nm against reagent blank and a calibration curve was constructed. The absorbance of the sample solution was measured, and the amount of TLB was determined by referring to the calibration curve or computed from the regression equation as illustrated in Figure 2 & 3.

**Figure 2: Absorption spectrum of TLB with BCG**



**Fig: 3: Standard Calibration Curve of TLB in Presence of BCG Reagent**



**2.3.3 Preparation of the sample solution**

Ten tablets of TLB were accurately weighed and powdered. Tablet powder equivalent to 100 mg of TLB was dissolved in 50 ml of methanol, sonicated for 15 mins and filtered. The filtrate is combined and the final volume was made to 100 ml with methanol for the above method. The solution was suitably diluted and analyzed as given under the assay procedure for bulk sample and the linearity absorbance range observed for TLB was shown in Table 1. The analysis procedure was repeated three times with tablet formulations and the result of analysis was determined as depicted and shown in Table 2.

**Table 1: Linearity absorbance range of TLB**

Concentration(µg/mL)	Absorbance
0.1	0.14
0.2	0.25
0.4	0.33
0.6	0.47
0.8	0.77

**2.4 Recovery Studies**

To ensure the accuracy and reproducibility of the results obtained, known amounts of the pure drug was added to the previously analyzed formulates samples and these samples were reanalyzed by the proposed method and also by performing recovery studies. The percentage recoveries, thus obtained for this method were given in Table 2.

**Table 2: Assay & recovery studies of TLB in tablet formulation**

Tablet Formulation	Amount Claim (mg/tablet)	*Amount obtained(mg) by the proposed method	**% Recovery by the proposed method
1	5	4.93	102.28
2	5	4.82	101.19
3	5	4.87	100.83

\*Average of three determinations

\*\*After spiking the sample

### 3. Results And Discussions

The extractive spectrophotometric methods are more popular due to their sensitivity in assay of the drug and hence, ion pair extractive spectrophotometric methods have gained considerable attention for quantitative determination of many pharmaceutical preparations. These proposed methods are extractive spectrophotometric methods for the determination of TLB by using chloroform as a solvent form in its formulation i.e. tablets. The color ion-pair association complexes formed is very stable. The working conditions of this method were established by varying one parameter at a time and keeping the other parameters fixed by observing the effect produced on the absorbance of the color species. Various parameters involved for maximum color development for this method were optimized. The proposed methods were validated statistically and by recovery studies. The molar absorptivity and Sandell's sensitivity values show the sensitivity of methods while the precision was confirmed by % RSD (Relative Standard Deviation). The optical characteristics such as absorption maxima (nm), molar absorptivity ( $\text{lit. mol}^{-1} \text{cm}^{-1}$ ), correlation coefficient (r) and Sandell's sensitivity ( $\text{mg}/\text{cm}^2/0.001$ ) were calculated and are also summarized in **Table 3**. Assay results of recovery studies are given in **Table 2**.

Results are in good agreement with labeled values. The percent recovery obtained indicates non-interference from the common excipients used in the formulation. The reproducibility, repeatability and accuracy of these methods were found to be good, which is evidenced by low standard deviation. The proposed method is simple, sensitive, accurate, precise and reproducible. These are directly applied to the drug to form chromogen. Hence, they can be successfully applied for the routine estimation of TLB in bulk and pharmaceutical dosage form even at very low concentration and determination of stability of drug in formulation such as tablets.

**Table 3: Optical Characteristics and Regression Analysis of the Proposed Method**

Parameters	Proposed method
Measured $\lambda_{\text{max}}$ (nm)	624
Beers law limit ( $\mu\text{g}/\text{mL}$ )	0.1-0.8
Molar absorptivity ( $\text{L}/\text{mole}/\text{cm}$ )	$0.43 \times 10^4$
Sandell's sensitivity ( $\text{mcg}/\text{cm}^2/0.001$ Absorbance unit)	0.7674
Regression Equation ( $Y = mx + c$ )	$Y (0.0309) = 1.065x + 0.0396$
Slope (m)	1.065
Intercept (c)	0.0309
Standard Error of Estimate	0.0396
Correlation coefficient (r)	0.994
Precision (% Relative standard deviation)	0.3068
Color stability (hours)	1hr 30 min
Confidence intervals (upper limit =1)	0.909-0.794
LOD ( $\mu\text{g}/\text{ml}$ )	0.07
LOQ ( $\mu\text{g}/\text{ml}$ )	0.21

### 4. Conclusion

TLB was estimated successfully by the developed extractive spectrophotometric method, both as a pure compound and also the constituent of a tablet formulation. The method is simple, rapid, accurate, or cost-effective with high accuracy & precision and does not involve any critical reaction conditions, or tedious sample preparation. It is unaffected by slight variations in experimental conditions such as pH, dye concentration, shaking time and temperature. The applicability of the new procedure for routine quality control of TLB in pharmaceutical formulations was established.

The results of this labor of love are set forth by developing a simple, precise and accurate method for the estimation of TLB in bulk drug sample and also in its pharmaceutical dosage forms, used for routine analysis of TLB.

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